



National Wildlife Health Center



# Avian Influenza: Background, Current Situation, Detection

Jan 18, 2006. Anchorage, Alaska  
Hon S. Ip

**National Wildlife Health Center**  
*Advancing wildlife and ecosystem health*

# Roadmap

Avian Influenza

Background

Current Situation

Detection

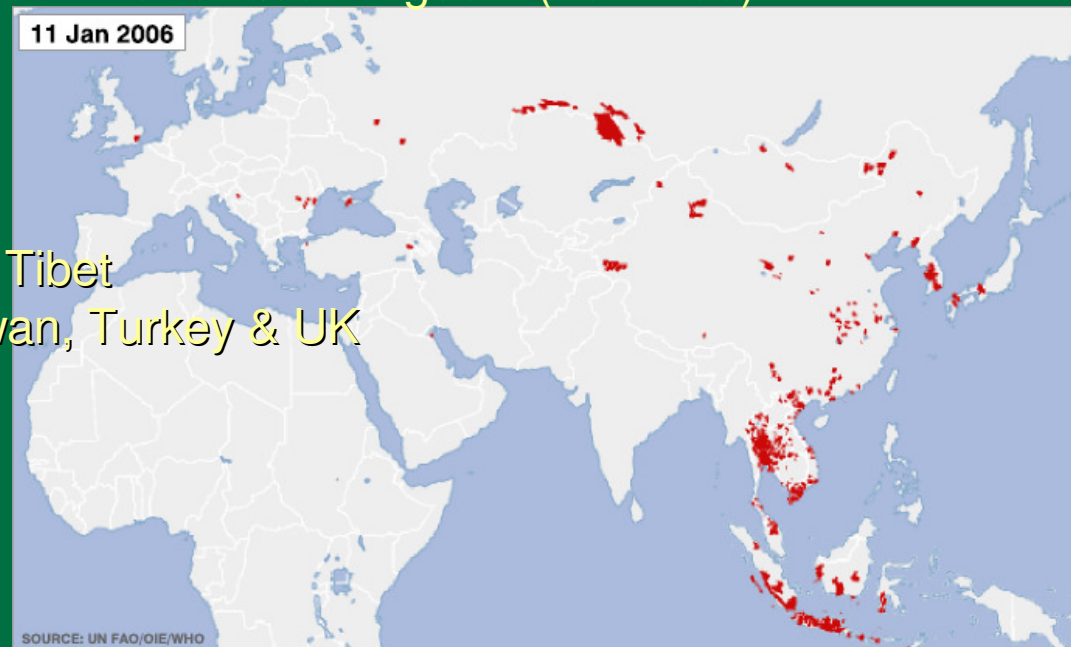


NMHM

# H5N1-Current Situation (Geographic)

1. Cambodia
2. China (People's Republic of)  
Hong Kong (SAR)
3. Indonesia
4. Croatia (10/25/05)
5. Japan
6. Kazakhstan (8/1/05)
7. South Korea
8. Laos
9. Malaysia
10. Mongolia (8/10/05)
11. Philippines (H5-retracted)
12. Romania (10/7/05)
13. Russia (7/21/05)
14. Taiwan (10/05 smuggled birds)
15. Tibet (8/1/05)
16. Turkey (10/13/05)
17. Thailand
18. Vietnam
19. Ukraine (11/25/05)
20. United Kingdom (10/22/05)

- May –Qinghai
- July – Russia
- August - Kazakhstan, Mongolia & Tibet
- October – Croatia, Romania, Taiwan, Turkey & UK
- November – China, Ukraine
- Jan - Turkey



# H5N1-Current Situation (Human)

## H5N1 Human case count

Between 12/3/2003 as of 1/18/06

Country	Cases	Deaths
Vietnam	93	42
Thailand	22	14
Cambodia	4	4
Indonesia	17	12
China	8	5
Turkey	4	2
Total	148	79

- 53% mortality rate

# Roadmap

Avian Influenza

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# Roadmap

Avian Influenza

Background

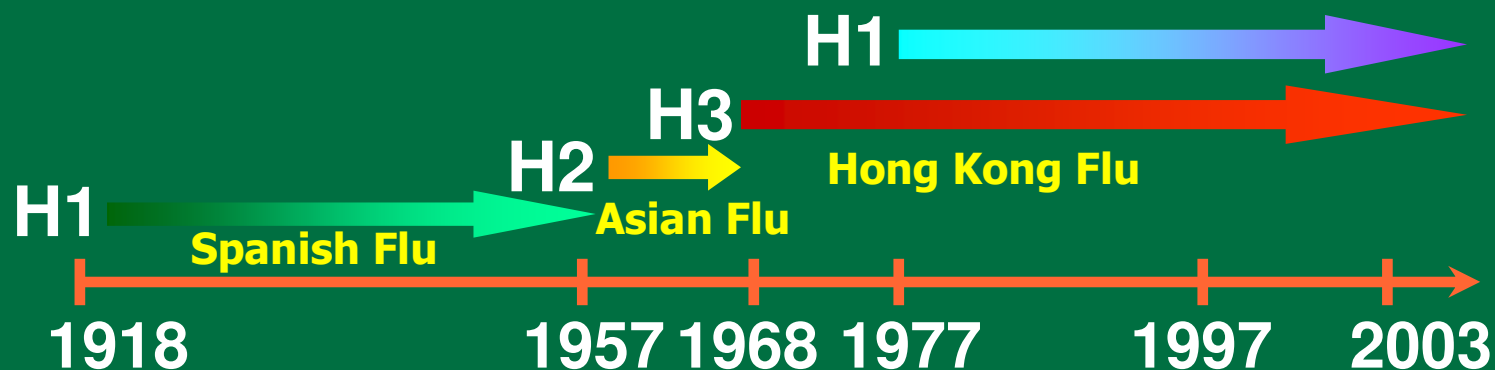
Pandemics

Current Situation

Detection

# Influenza Pandemics

- An epidemic that spread beyond a region or continent
- Usually accepted as the emergence of a new HA or NA serotype
- 1918-1919. Spanish Flu H1N1
- 1957-1958. Asian Flu H2N2
- 1968-1969. Hong Kong Flu H3N2





## Influenza Pandemics in the 20<sup>th</sup> Century



**1918: “Spanish Flu”**

> 20 million deaths

H1N1



**1957: “Asian Flu”**

1 million deaths

H2N2



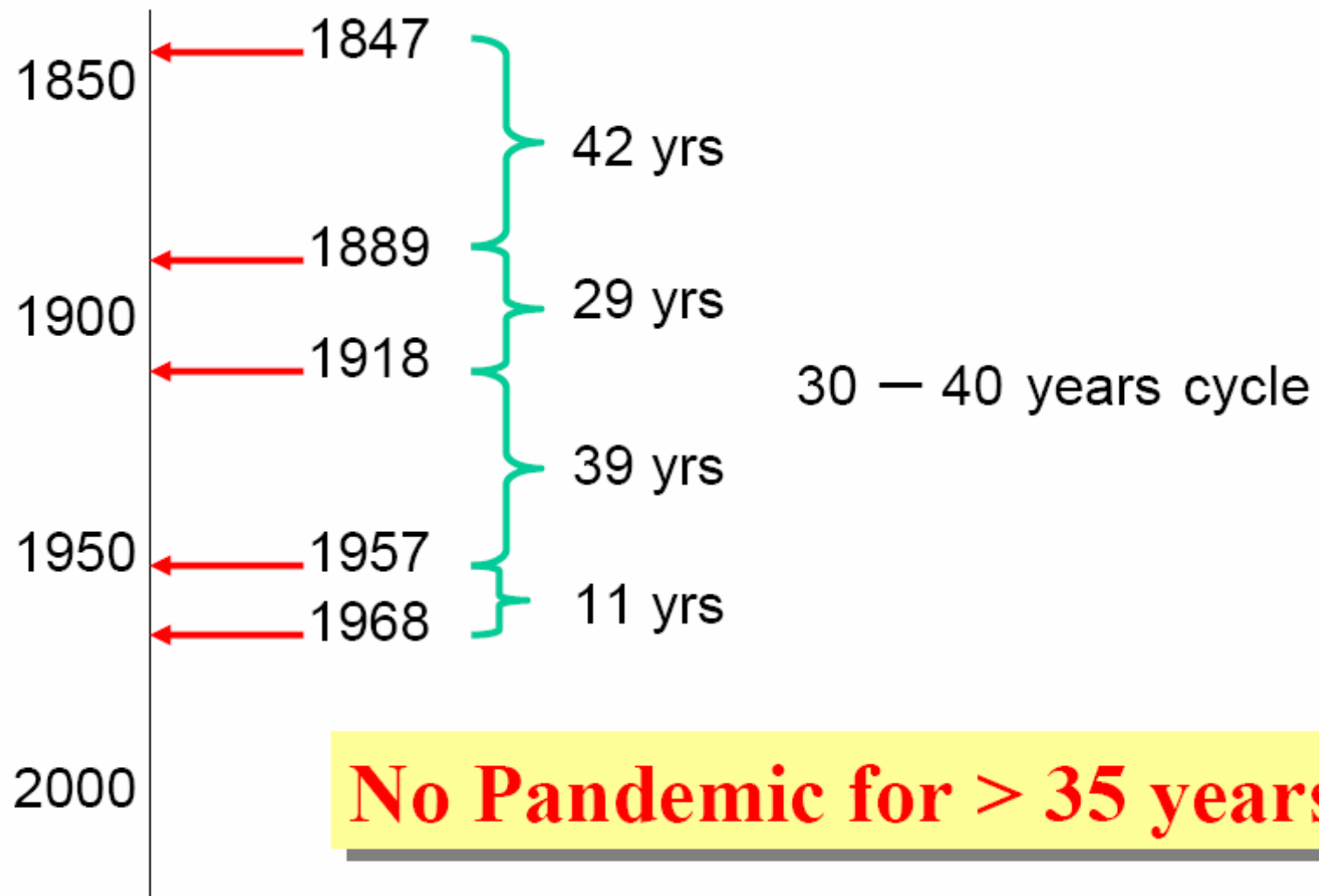
**1968: “Hong Kong Flu”**

1 million deaths

H3N2



# Past Influenza Pandemics



# Roadmap

Avian Influenza

Background

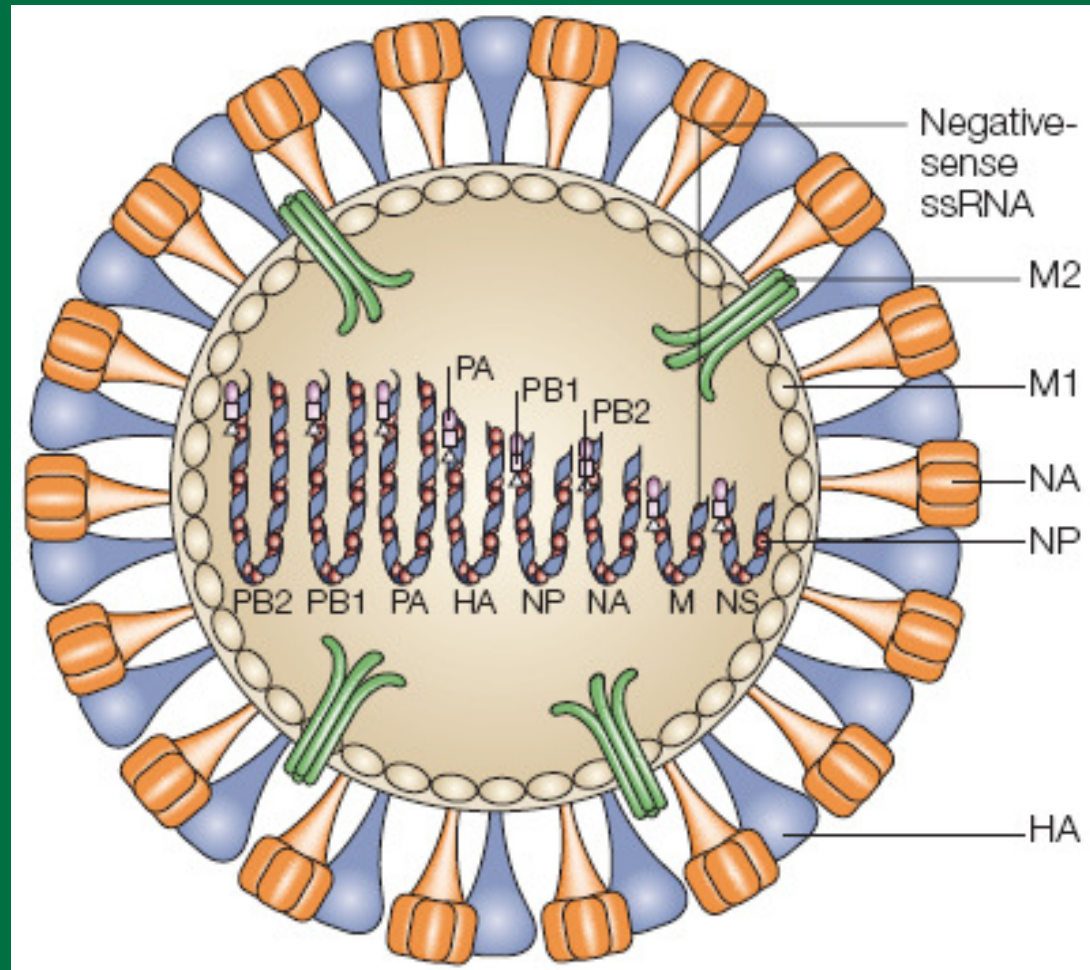
Pandemics

Influenza Viruses

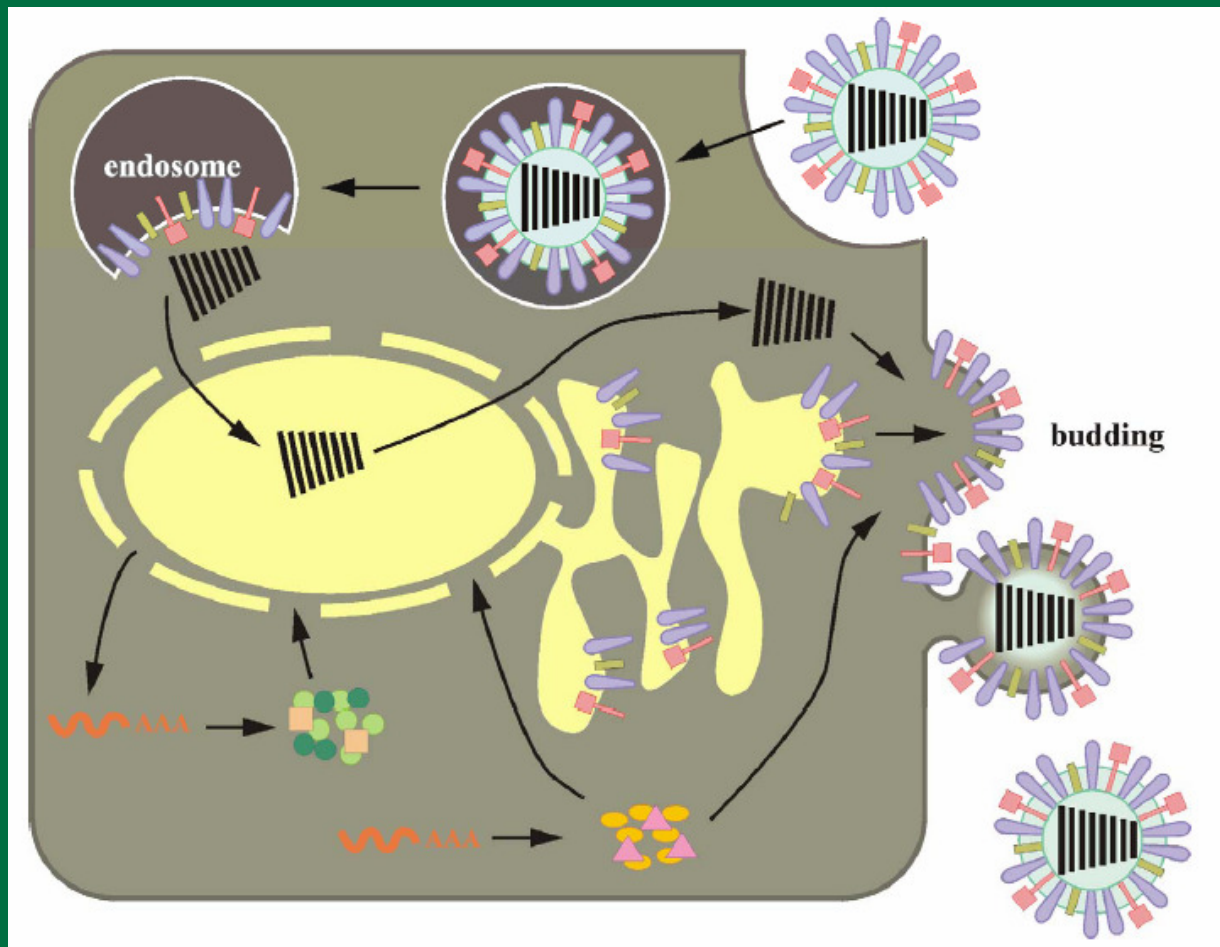
Current Situation

Detection

# Influenza Virus - Schematic



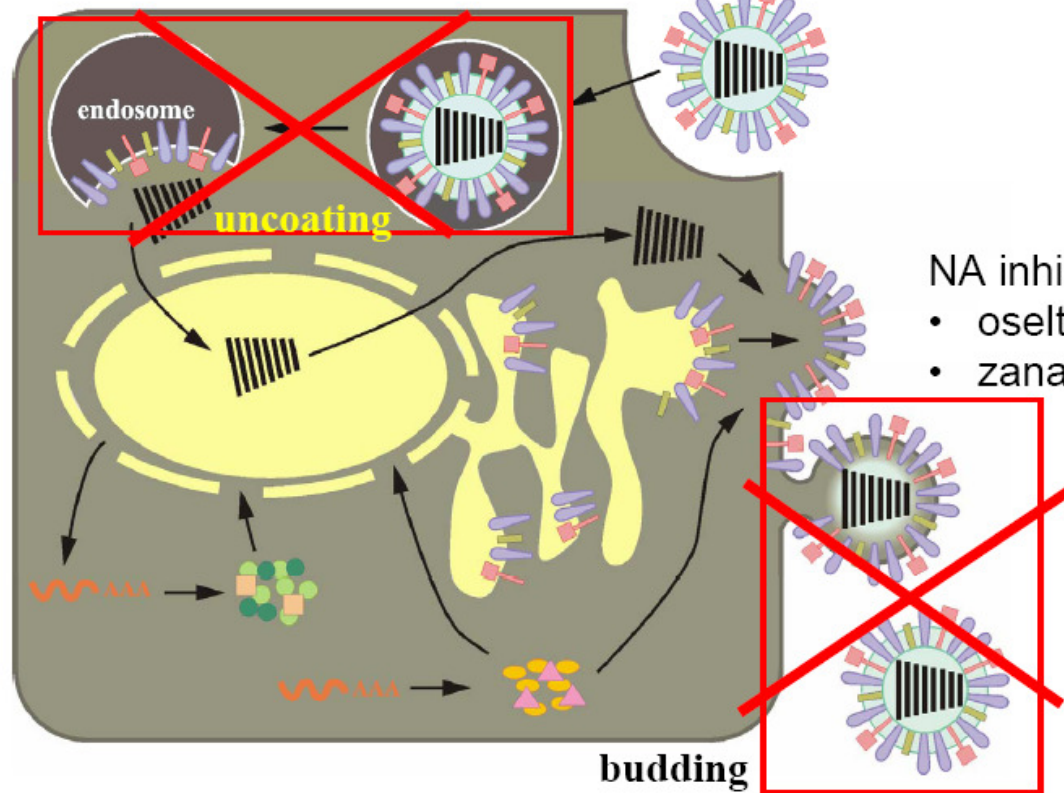
# Influenza Virus - Replication



# Influenza Virus - Antivirals

M2 inhibitors  
• amantadine and rimantadine

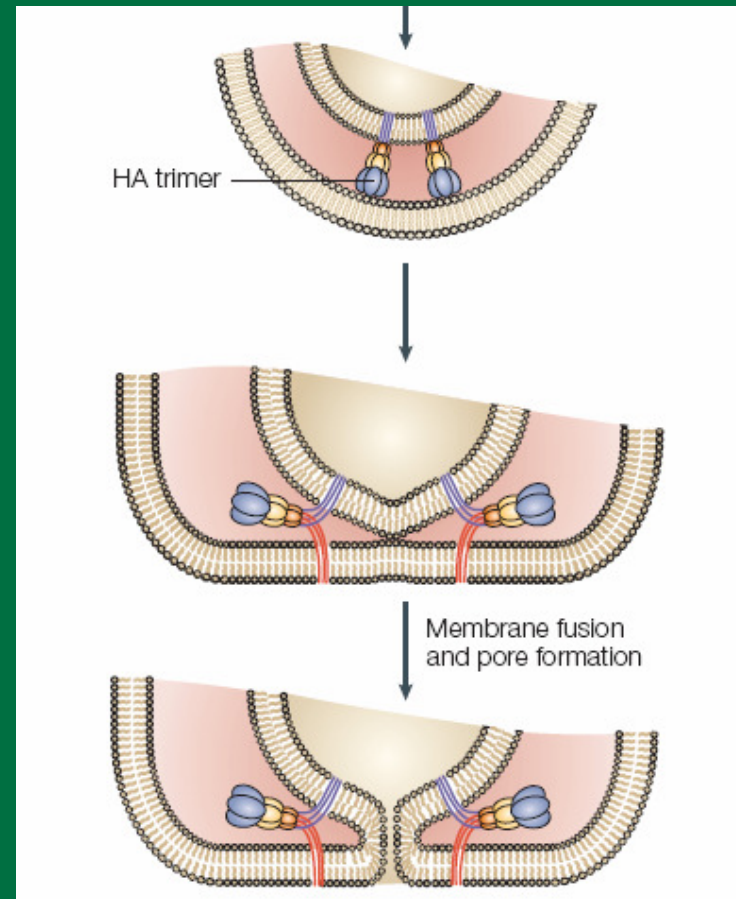
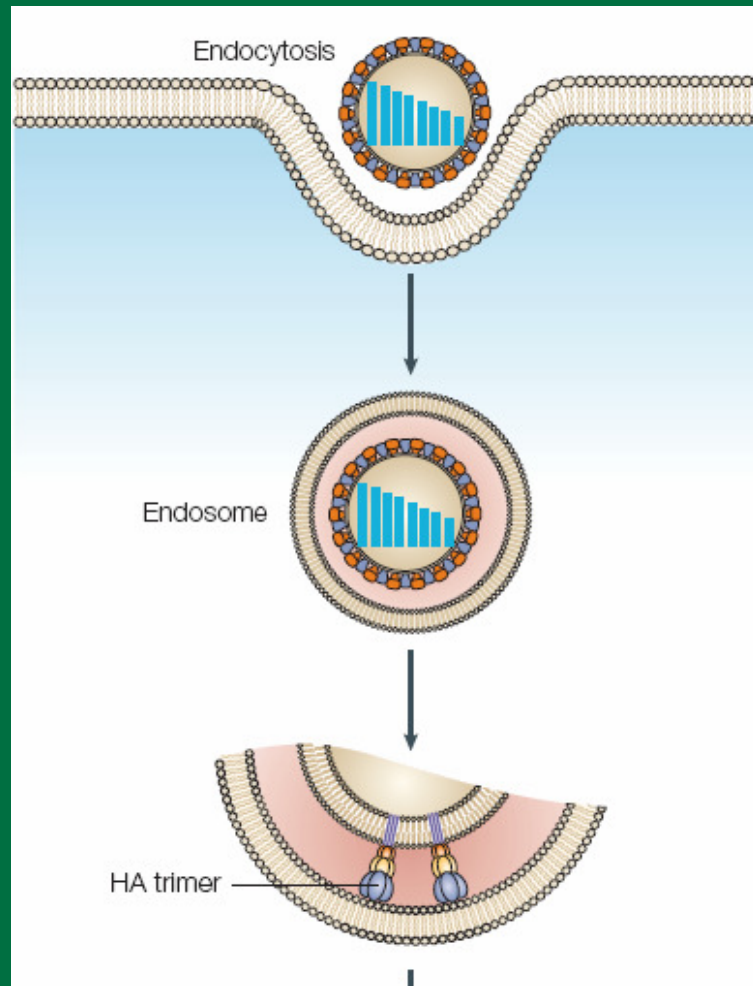
## Anti-influenza drugs



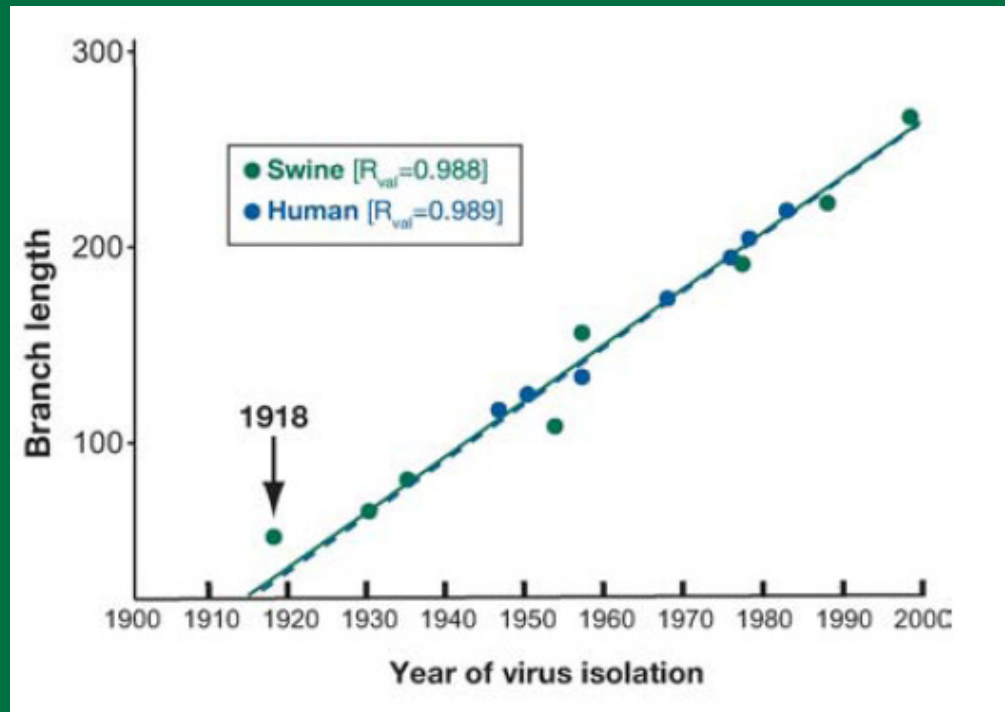
NA inhibitors  
• oseltamivir  
• zanamivir



# Hemagglutinin-induced Membrane Fusion



# Influenza – Genetic Drifts

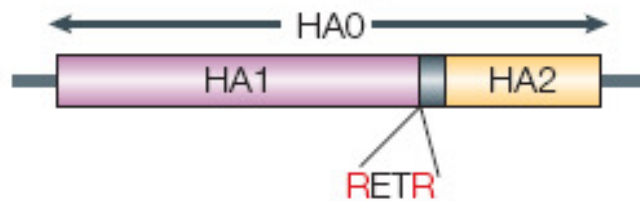
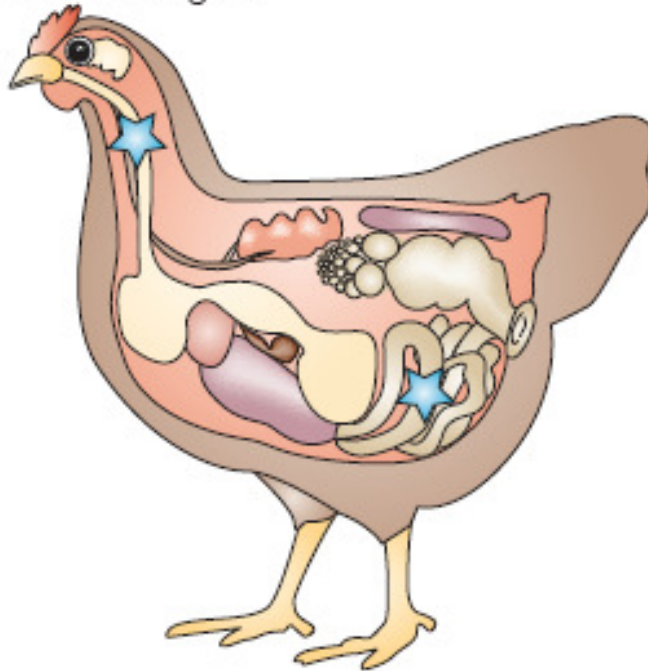




# Influenza Virus Hemagglutinin

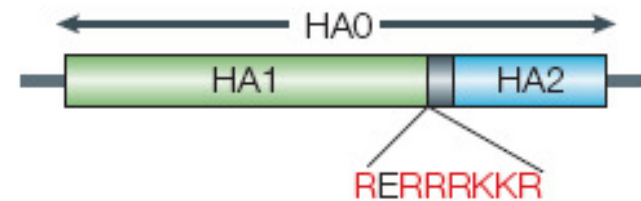
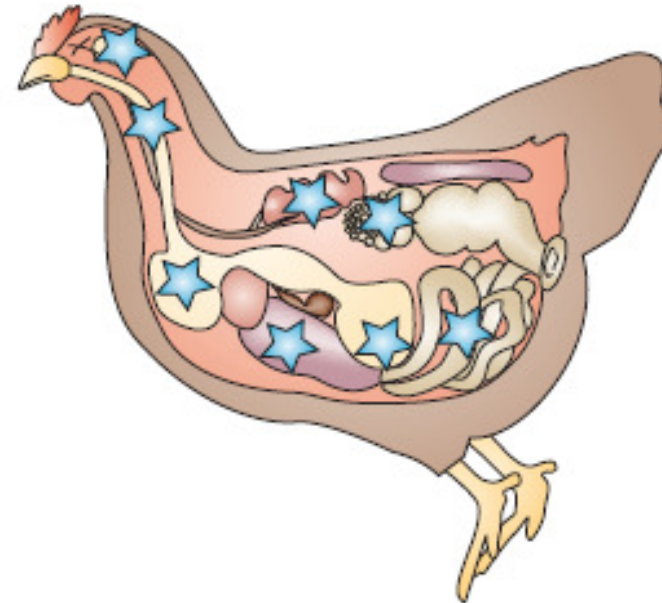
## LPAI

Proteases localized in respiratory and intestinal organs



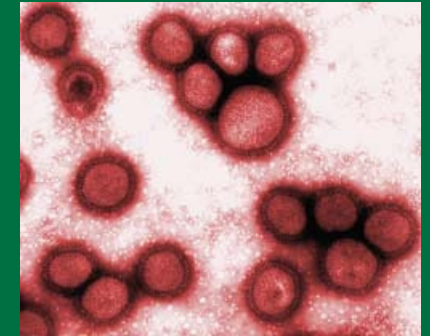
## HPAI

Ubiquitous proteases



# 1918 Virus is an Avian Virus

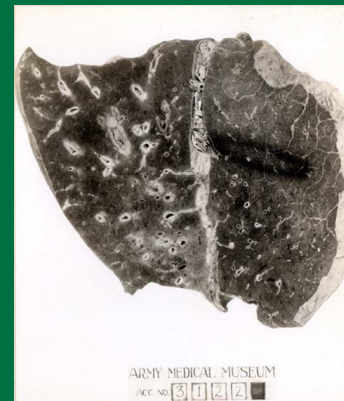
- Archived tissue and victim buried in permafrost
- A/Brevig Mission/1918 (H1N1)
- An avian virus that was adapted to humans
- Not a reassortant (vis 1957, 1968)
- 25 amino acid changes from avian sequence
- Some of these changes are already in the H5N1 virus



Kawaoka

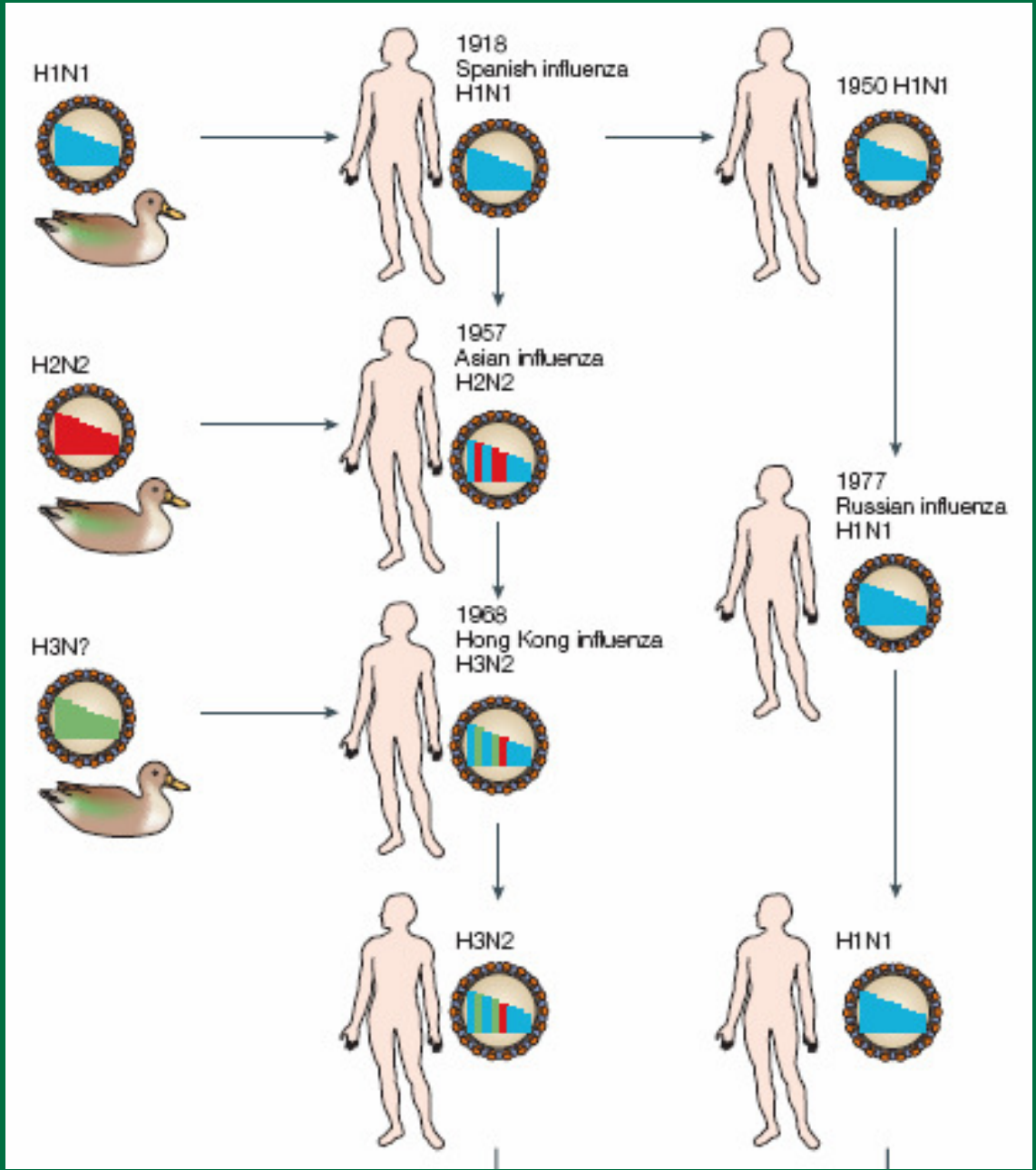


Ned Rozell



National Wildlife Health Center

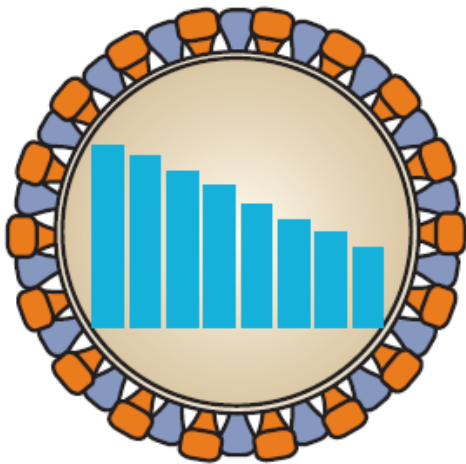
# Human Pandemic Influenza Viruses



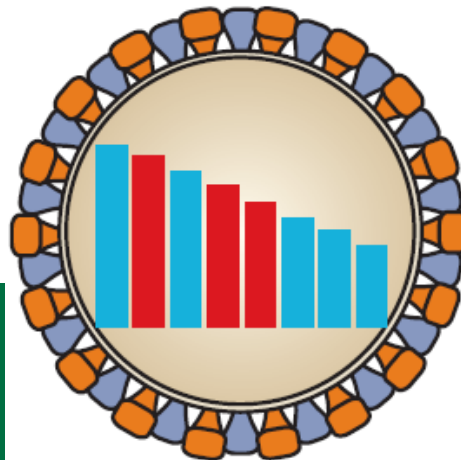
# All Pandemic Viruses have Avian Genes



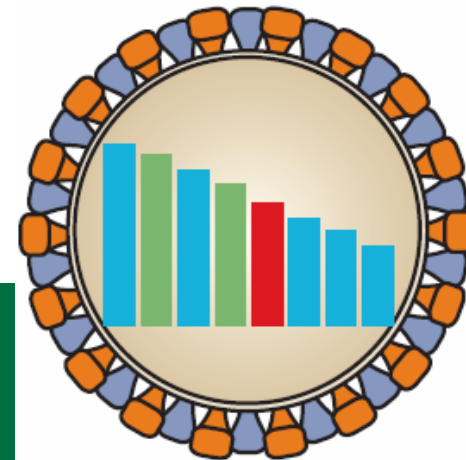
1918: "Spanish Flu"



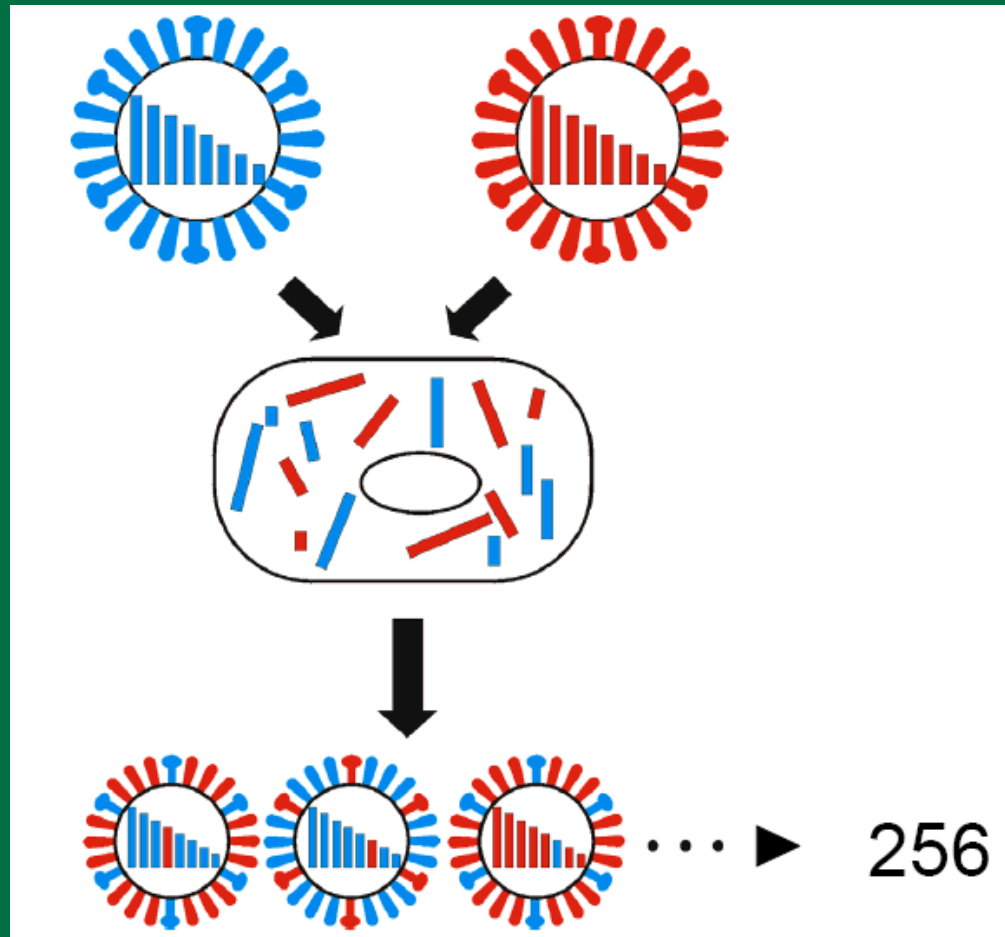
1957: "Asian Flu"



1968: "Hong Kong Flu"

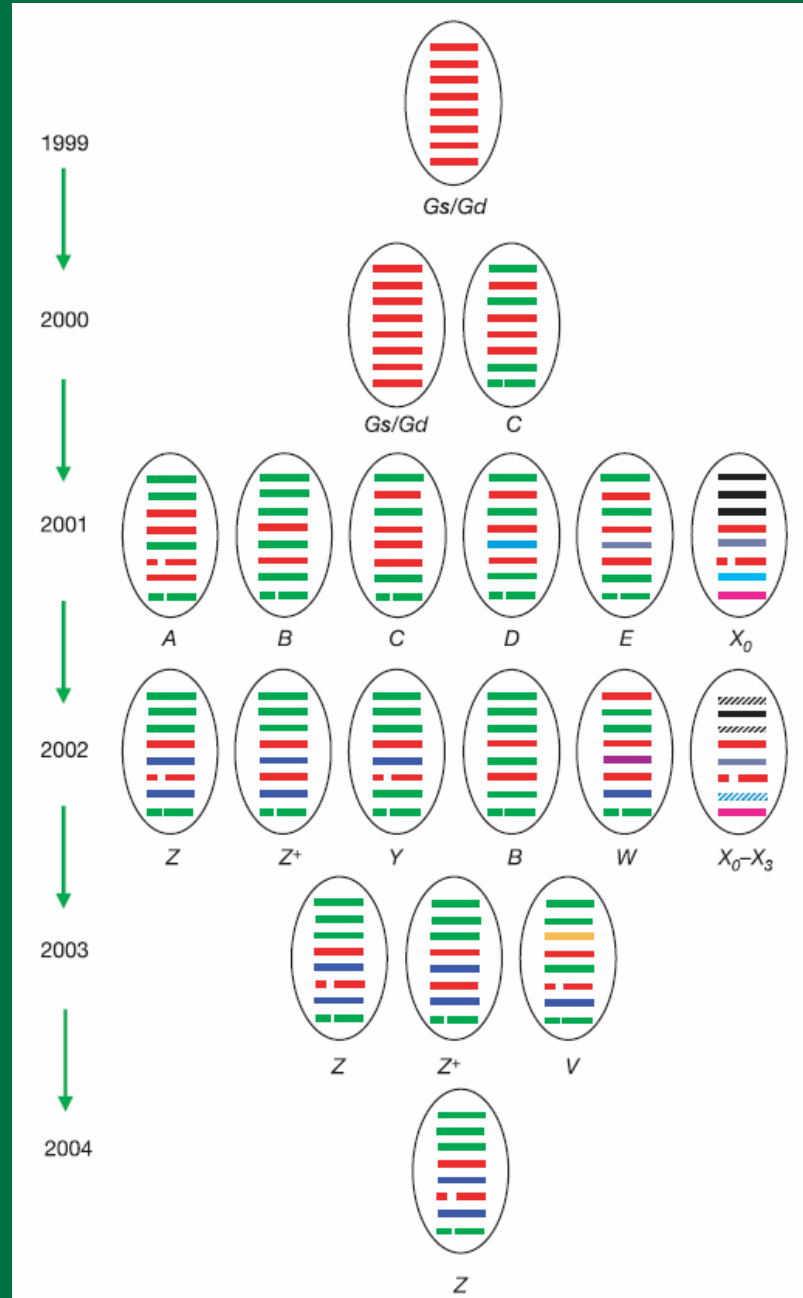


# Influenza – Genetic Shifts



Source: Kawaoak 2005.

# Evolution of H5N1



Source:



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# Roadmap

Avian Influenza

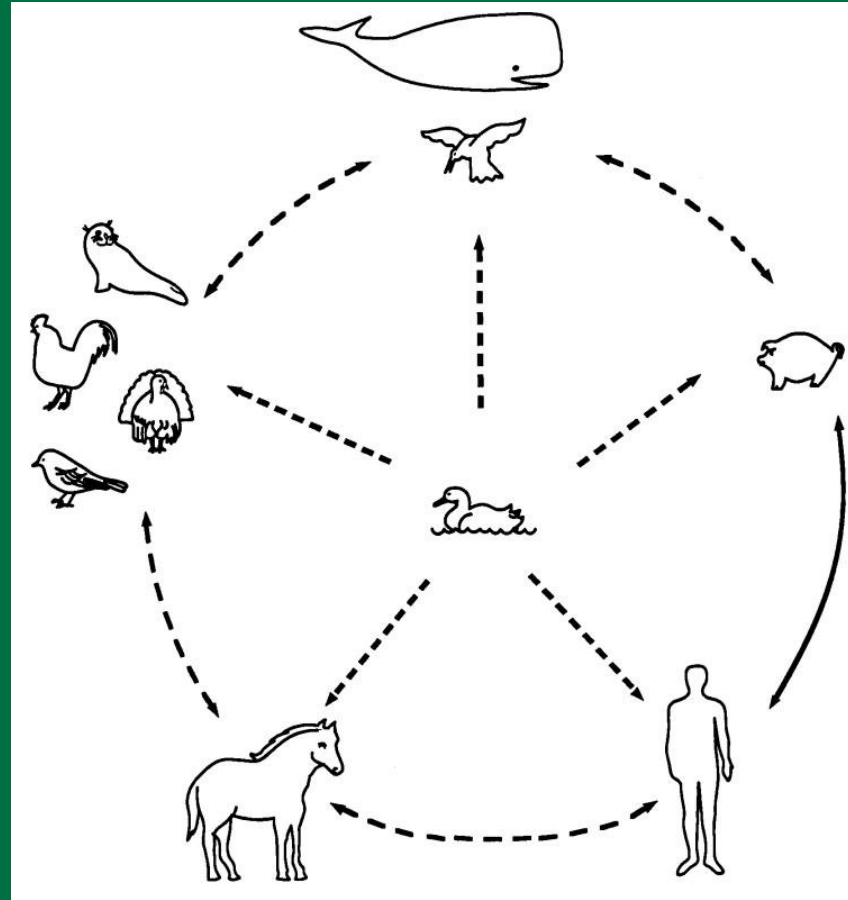
Background

Current Situation

Detection



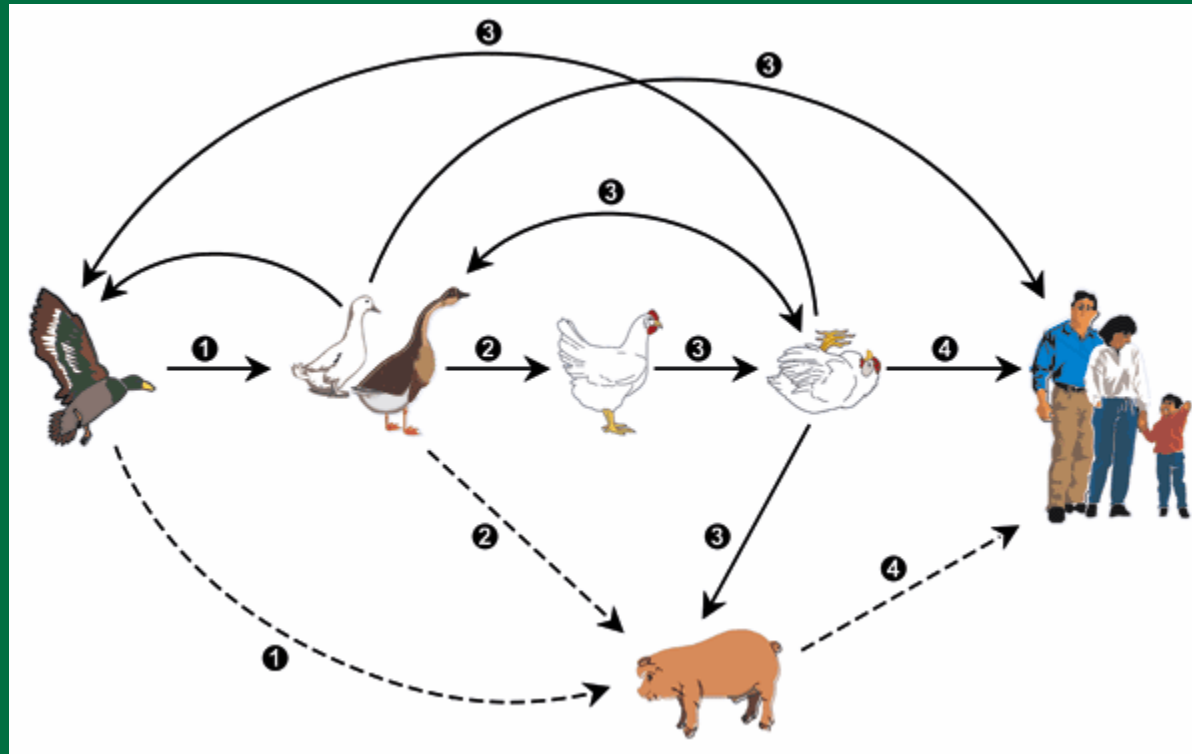
# Waterfowl and Avian Influenza



Waterbirds (ducks, gulls and shorebirds) are the natural reservoir



# Waterfowl and Avian Influenza



Webster's (2006) new paradigm for H5N1

# HPAI H5N1 in perspective

- Since 1959 there have been 24 major HPAI outbreaks
  - All except for H5N1 have been controlled by “stamping out”
  - 23 million head of poultry involved
  - ~400 human cases, 1 death
- 
- Since 1999, over 200 million birds have died or been culled in an attempt to control H5N1
  - 116 cases, 60 deaths



ZonaEuropa

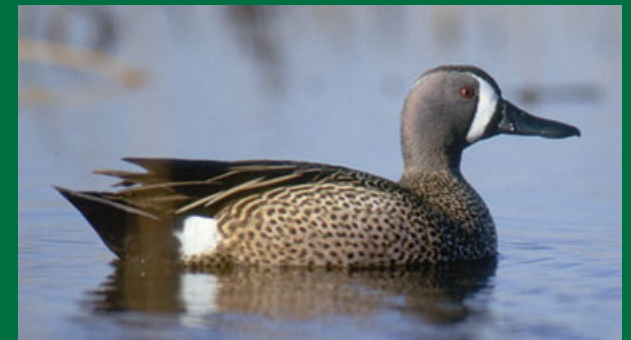
# Avian Influenza in Waterfowl

- All 16 HA and 9 NA combinations are present
- Subclinical/mild disease-respiratory/GI tract
- Fecal-oral transmission route
- No separate HPAI lineages
- AIV is in evolutionary “stasis” in waterfowl

H6 viruses isolated 81 years apart were 95.3% identical



Takashi Koike



David Stimac  
National Wildlife Health Center

# Role of Migratory Birds - Historical

- US. Extensive survey of wild birds during the 1983-1984 PA outbreak. 1/4466 samples positive.
- Australia. Of the five HPAI outbreaks in Australia, none has been associated with wild birds. Starlings were infected in the 1985 outbreak, but were associated with farms.
- New Zealand has never had an outbreak coincident with Australia.
- Europe. Close LPAI relative could be found in mallards for each HPAI outbreak in Europe since 1997 (Munster et al. 2005).

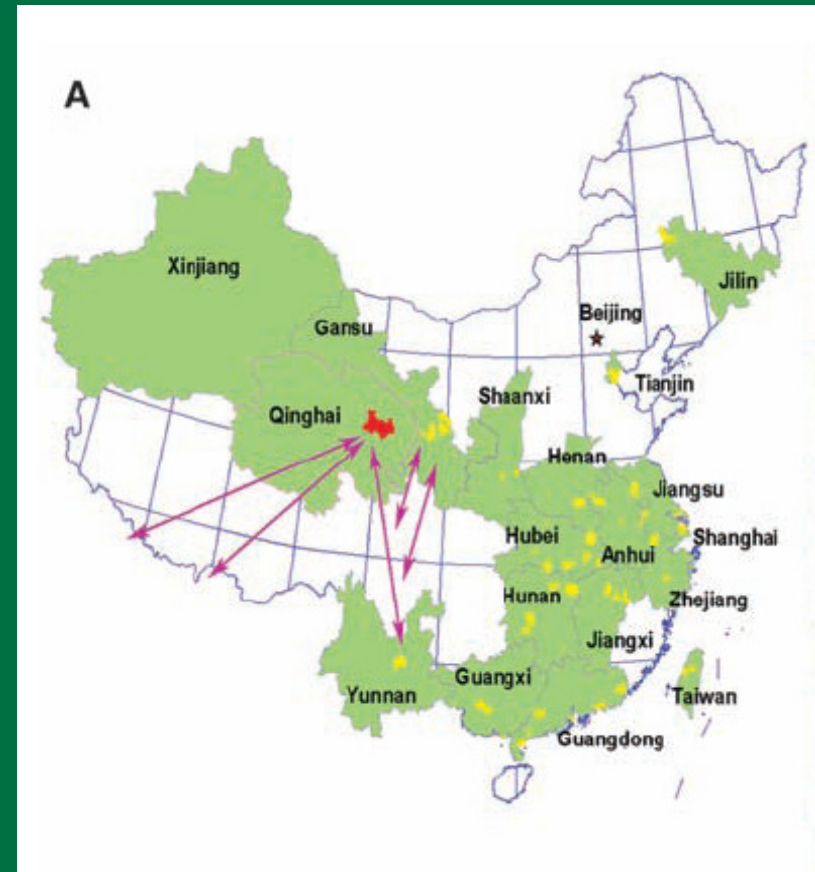
# H5N1 Outbreaks – 1997

- March. Poultry infected.
- May. First human case in a 3-year-old boy.
- August. Diagnosis of H5N1.
- November. Second human case.
- December. Isolation of H5N1 from poultry.
- Dec. 28. Island-wide culling implemented.  
1000 workers  
160 farms, 1000 LBMs.  
1.5 million chickens.



# Qinghai Outbreak-May 2005

- Wild birds reported affected at Qinghai Nature Reserve
- Mainly bar-headed geese (*Anser indicus*)
- Gulls (2 spp.)



Source: Liu et al, 2005. Science.

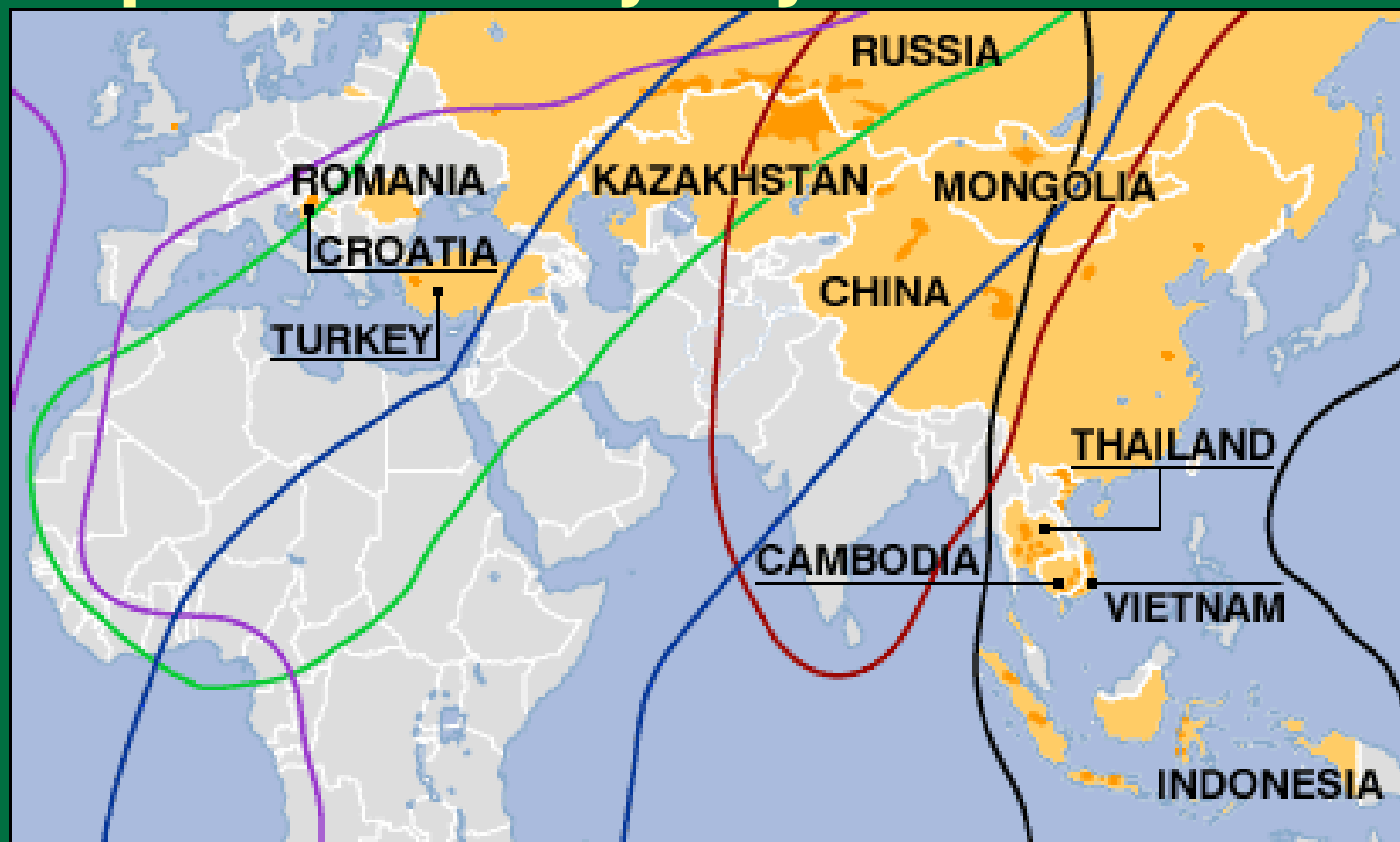


# H5N1 Geographical Spread – China

- Oct 19, 2005. Tengiyang, Hohhot, Inner Mongolia.
- Oct 25, 2005. Tianchang, Anhui Province.
- Oct 26, 2005. Wantang, Hunan Province.
- Nov 4, 2005. Badaohao, Liaoning Province.



# H5N1 Spread and Flyways



## MAIN MIGRATORY BIRD ROUTE BOUNDARIES

- Black Sea/Mediterranean
- Central Asia
- East Asia/Australian
- East Africa/West Asia
- East Atlantic

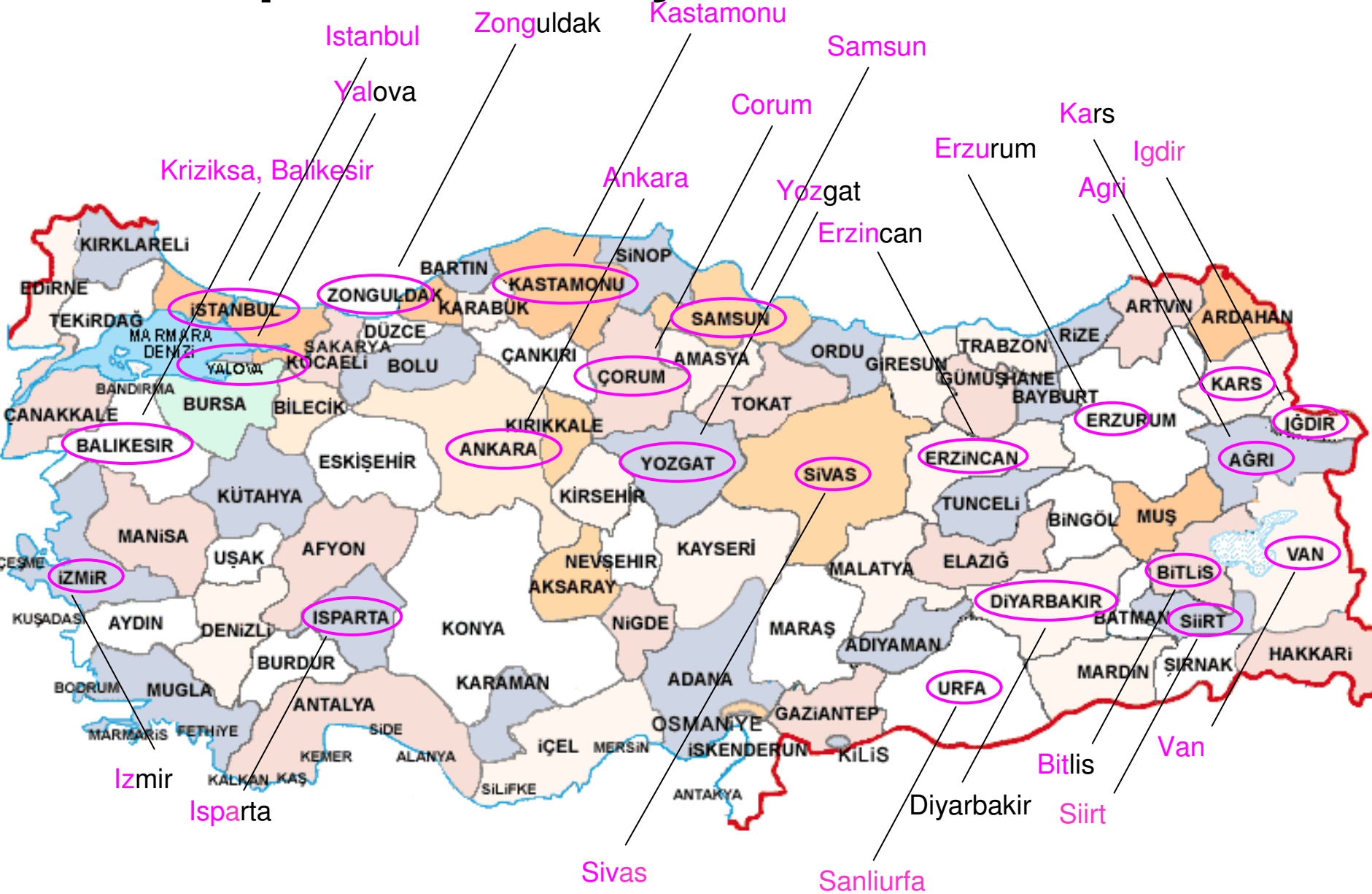
## COUNTRIES AFFECTED

- Locations of H5N1 outbreaks
- Countries with outbreaks

SOURCE: UN FAO/OIE



# H5N1 Spread –Turkey Jan 2006



# Turkey- As of 1/15/06



Baku



Saliurfa

Siirt

Cyprus

# Turkey- As of 1/15/06

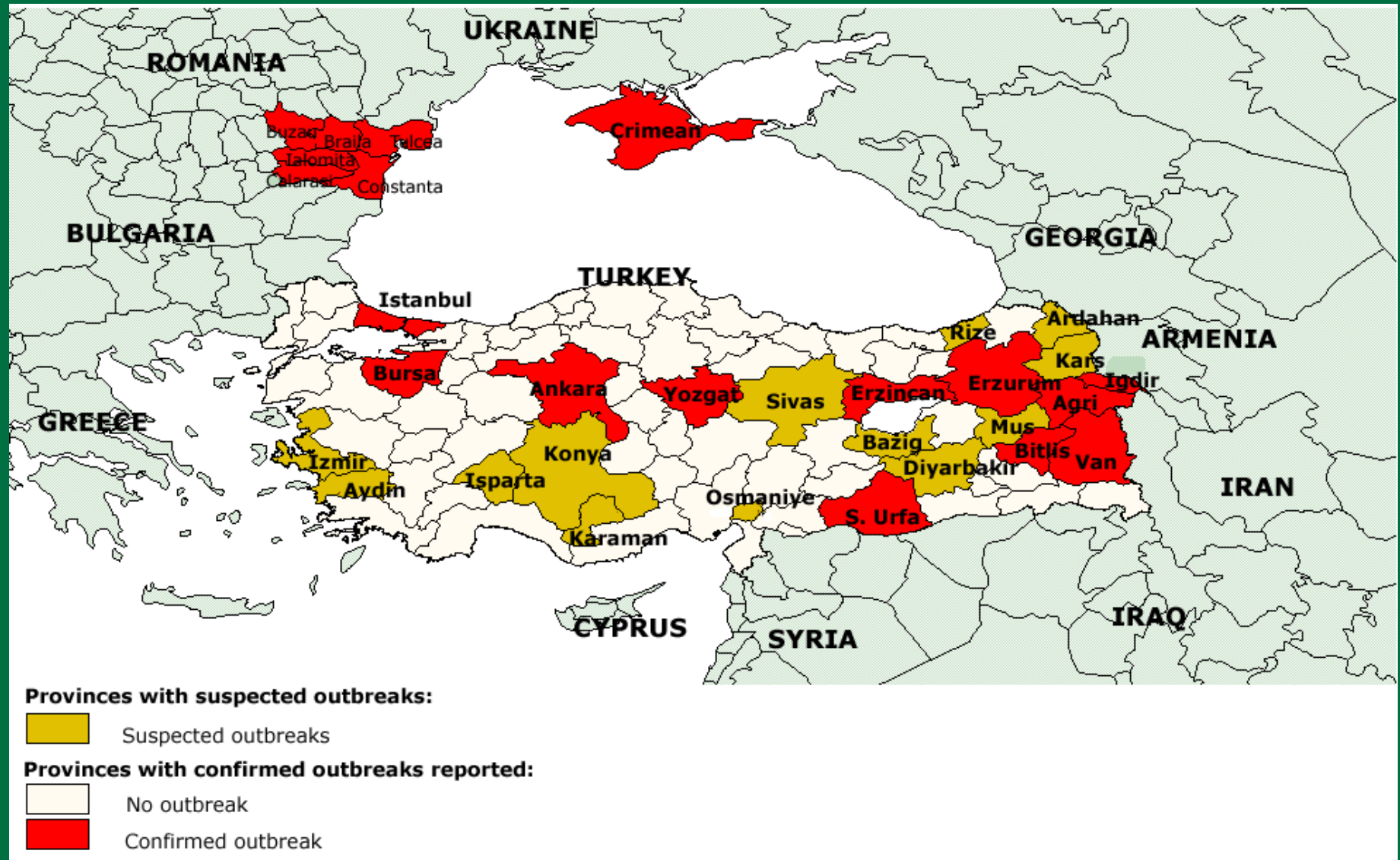
## POSITIVE AVIAN INFLUENZA CASES IN WILD BIRDS

Annex V

16.01.2006



# Turkey- As of 1/15/06





# Routes of Introduction to North America

- Infected Individuals
- Commercial Traffic
- Wild Birds



# Routes of Introduction to North America



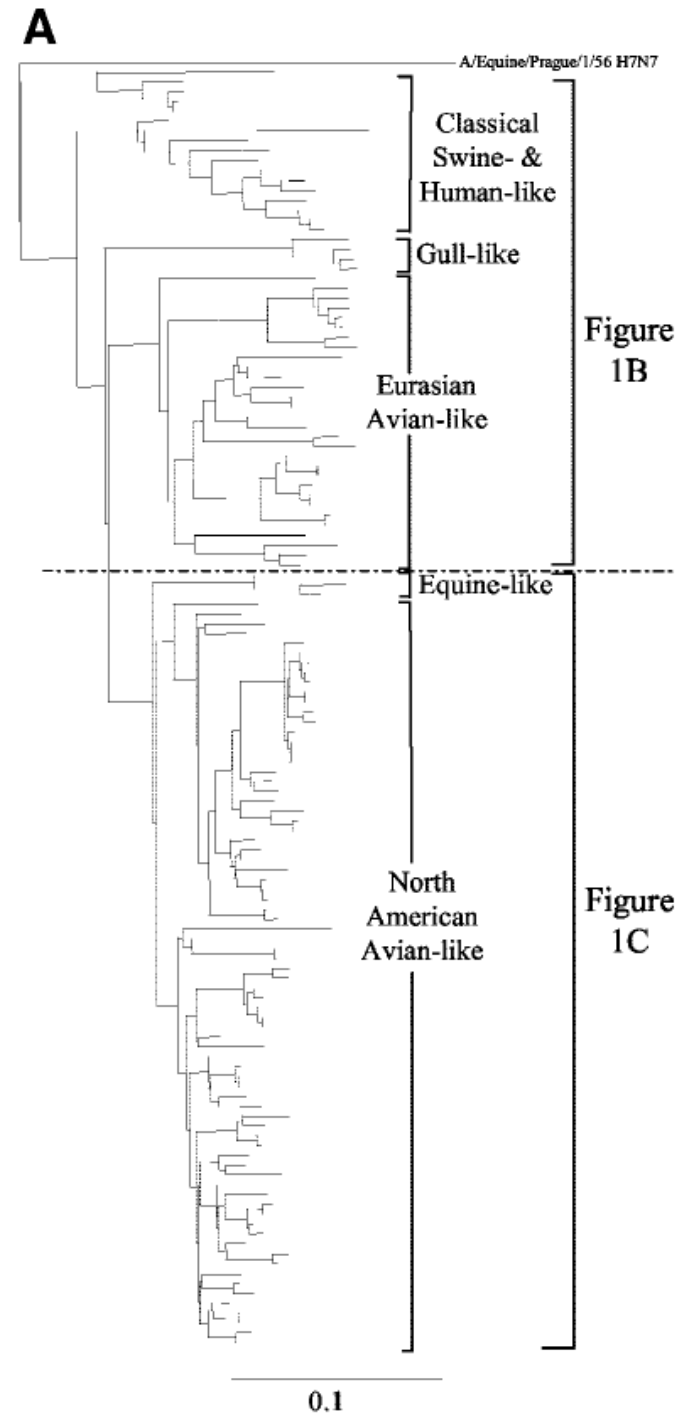
## Commercial Traffic

- Sept 05 – shipping container with 820 cases of eggs (120 eggs each) discovered on a ship in a California port without permits and not listed on ship's manifest
- Between Oct and Nov 2005, a total of 165,000 pounds of poultry products being smuggled into the US from Asia were intercepted
- Illegal products have been detected at markets and restaurants in New York City and Chicago

# Molecular Phylogeny Shows Intercontinental Contact

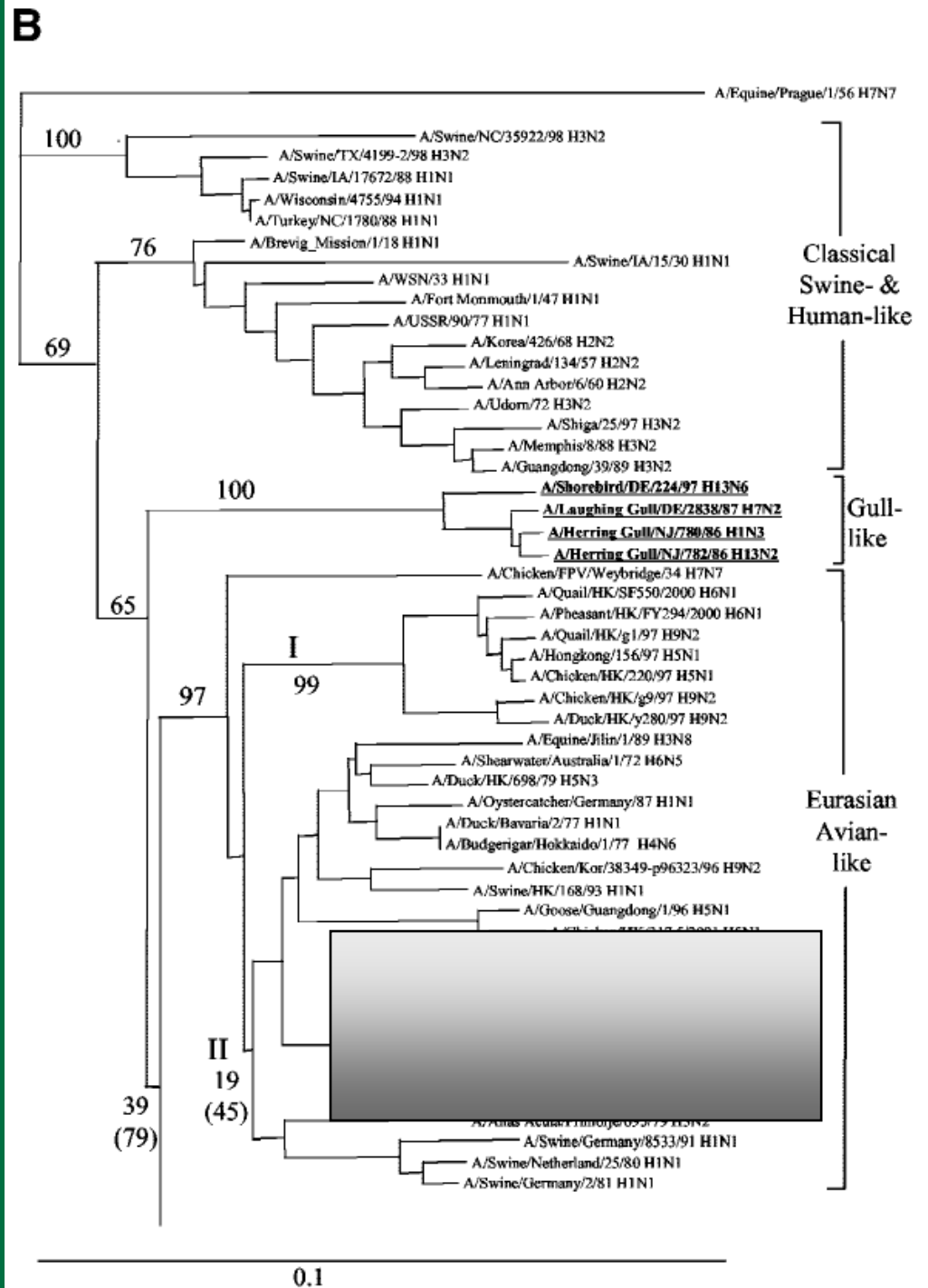
- Groups of influenza viruses cluster
- Avian viruses separate from human influenza viruses
- Distinct Eurasian/North American lineages of AIV

Source: Widjaja et al., 2004. J Virol 78:8771.



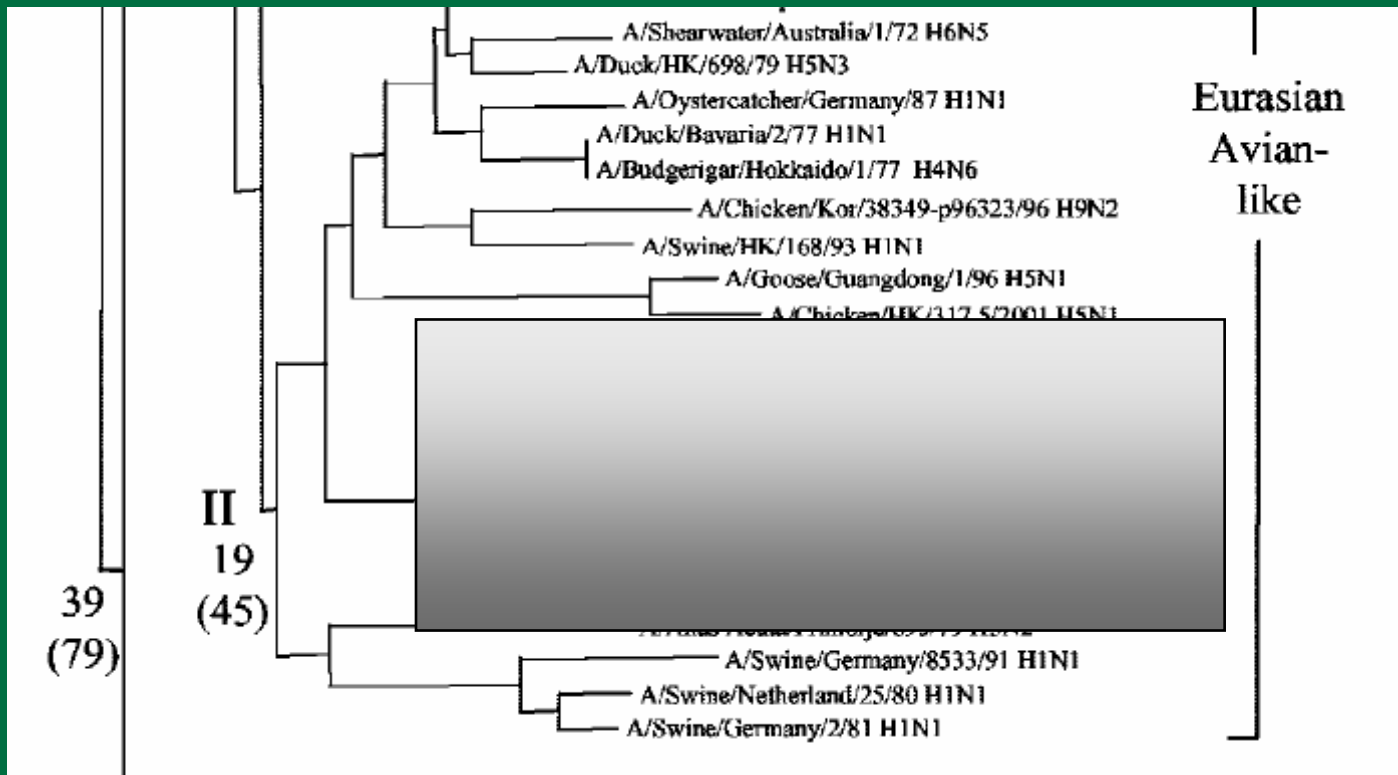
- Top part of figure from previous slide
- Influenza viruses from humans and swine form one cluster
- Avian cluster is divided into an anseriform and a gull group
- Enlarge bottom section

Source: Widjaja et al., 2004. J Virol 78:8771.





# Intercontinental Genetic Exchange



- East Coast North American isolates that are of Eurasian lineage

Source: Widjaja et al., 2004. J Virol 78:8771.

# H5N1 Isolates from Apparently Healthy Wild Birds

1. Crested hawk eagle (*Spizaetus nipalensis*; van Borm et al., 2005)
2. Great crested grebe (*Podiceps cristatus*; Lvov et al. 2005)
3. Bar-headed goose (*Anser indicus*; Ellis et al., 2004)
4. Canada goose (*Branta canadensis*; Ellis et al., 2004)
5. Tree sparrow (*Passer montanus*; Kou et al., 2005)

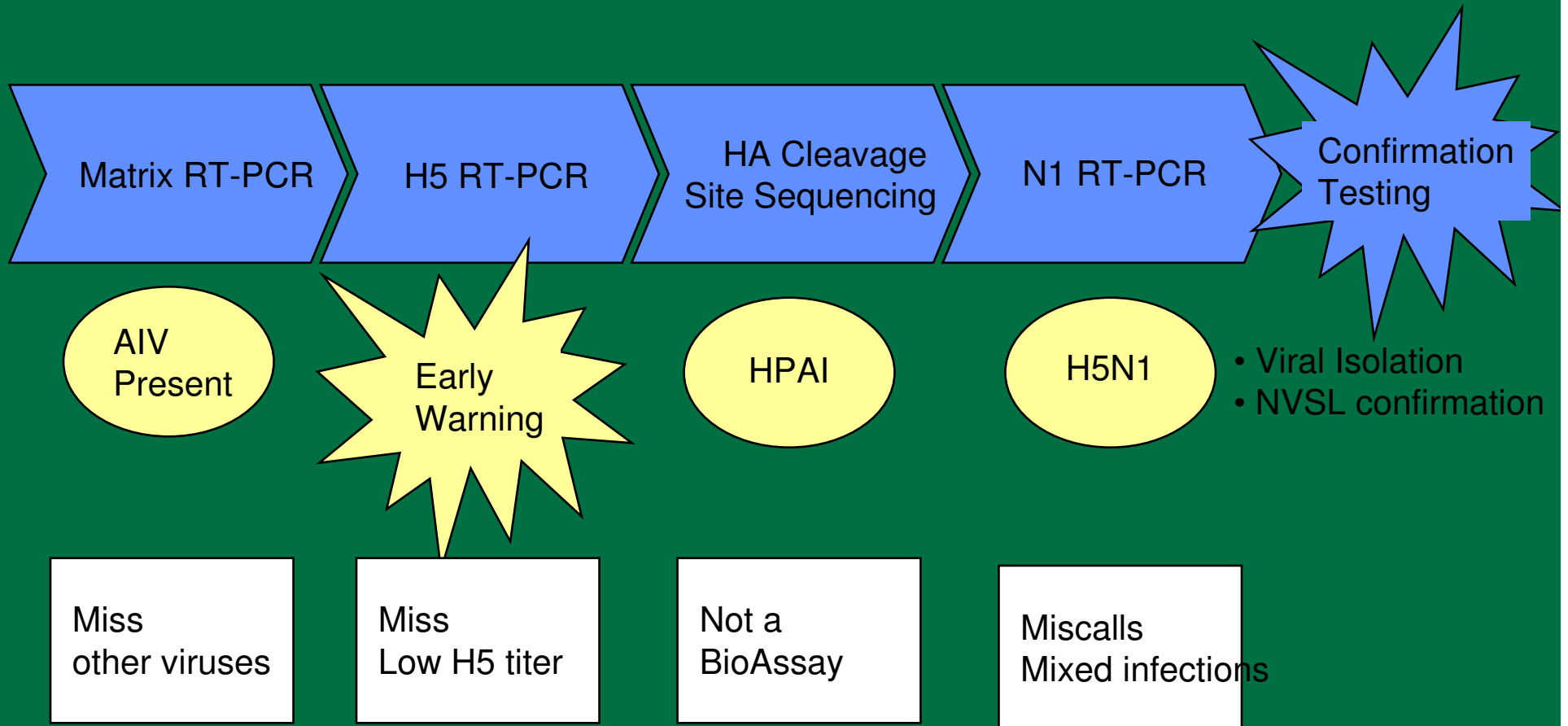
# Roadmap

Avian Influenza  
Background  
Current Situation  
Detection

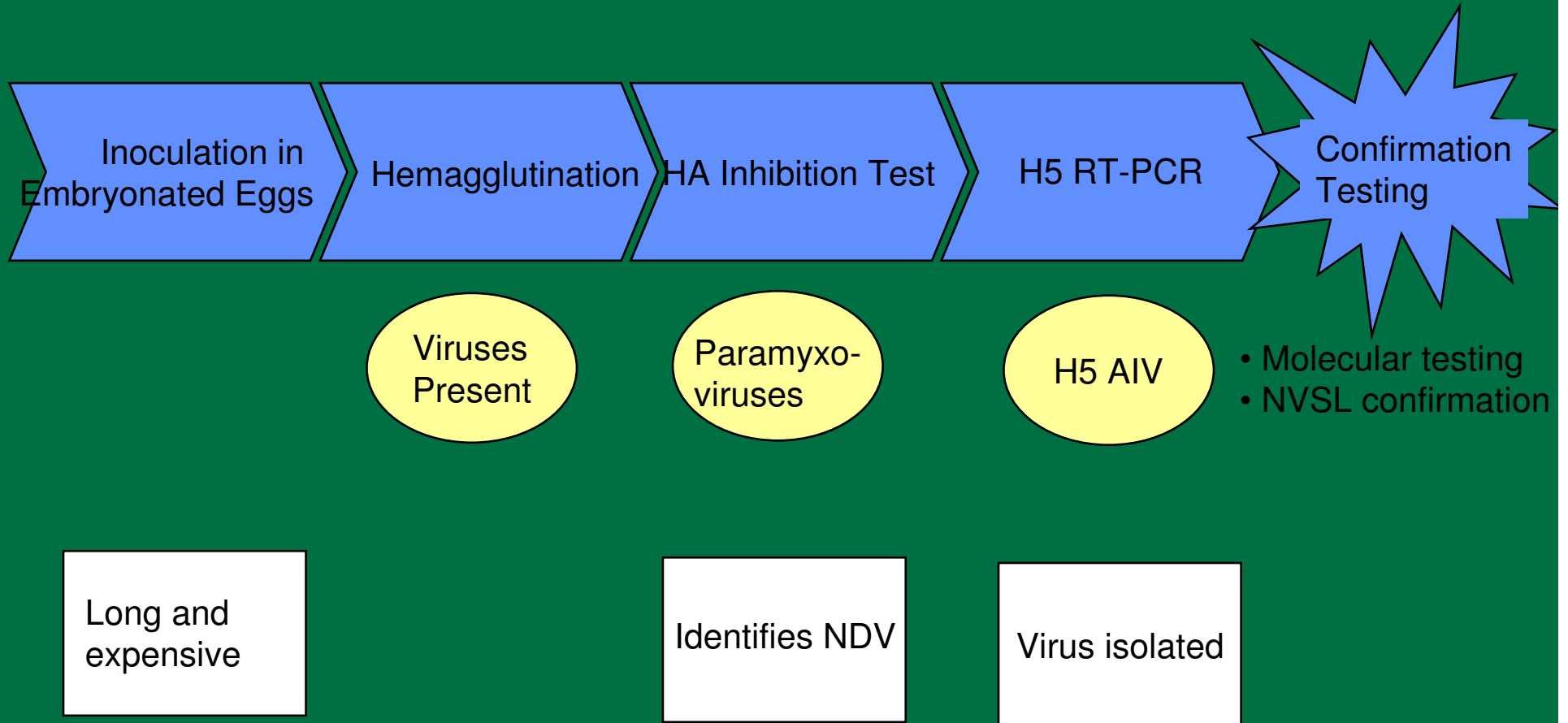
# Dual Approaches to H5N1 Testing

- Molecular Detection
  - Fast
  - Automatable
  - May not be as sensitive
  - Negatives not informative
- Viral Isolation
  - Gold standard, accepted
  - Definitive
  - Labor-intensive, slower
  - Isolates available for further study

# Approach 1- Molecular Testing



# Approach 2- Viral Isolation



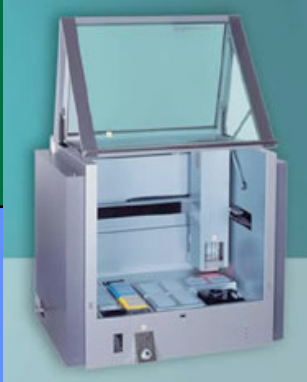
# Testing Panel

- Viral Isolation
  - Isolation in SPF Embryonated Egg
  - Hemagglutination Test - Chicken/Turkey RBC
  - HA Inhibition Test - APMV1, APMV2, APMV3
  - Subtyping by antibody panel (NVSL)
  - ICPI (NVSL)
- Molecular Testing
  - Matrix gene RT-PCR Test
  - North American Lineage H5 RT-PCR
  - Eurasian Lineage H5 RT-PCR
  - N1 RT-PCR
  - Whole Gene Cloning and Sequencing
  - HA Cleavage site PCR and Sequencing

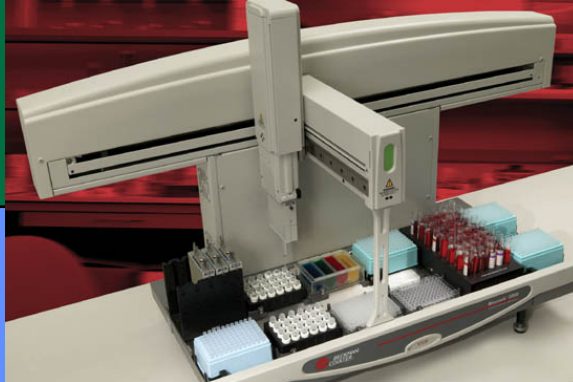


# Instrumentation

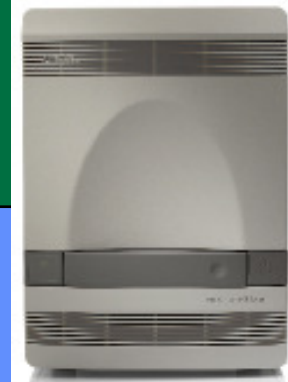
## Molecular Testing



Automated Sample Extractor

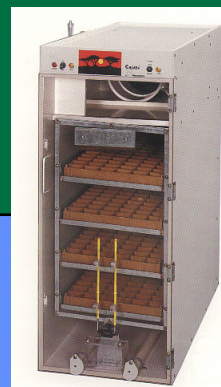
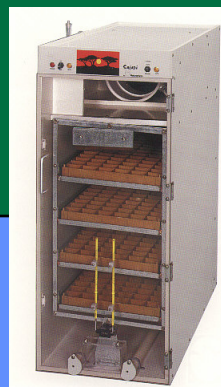
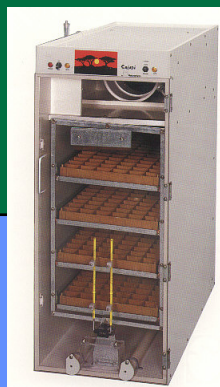


Robotic Molecular Biology Workstation



Real time Thermal cycler

## Viral Isolation

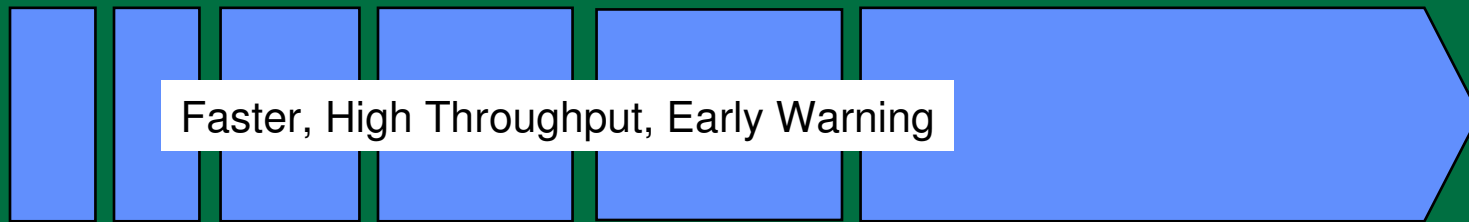


NatureForm Egg Incubators

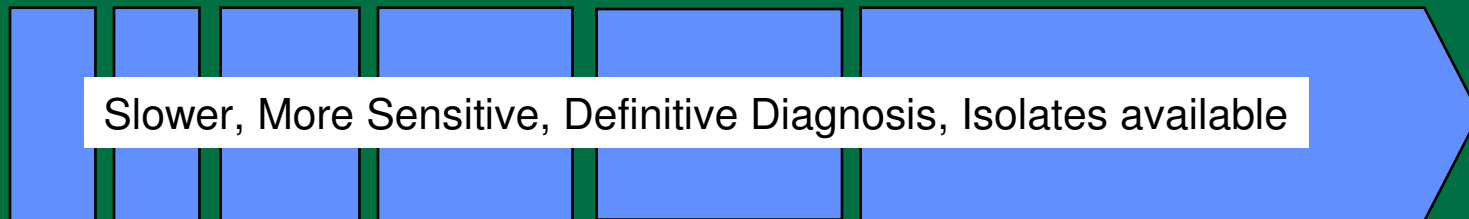


# Dual Approach - Benefits

## Molecular Testing



## Viral Isolation



# Summary of DVL AI Testing

1/13/06

Samples Received	1379
# Viruses Isolated	123
# Avian Influenza Viruses	59
# H5 AIV	0

# Comparison of Transport Media

	USDA AI medium	NWHC VTM medium
<b>Virus recovery in Eggs</b>		
Samples Received	100	100
Samples tested	92	91
No. Virus Isolated	14	22
<b>Percent Positive (HA)</b>	<b>15.2%</b>	<b>24.2%</b>
<b>Detection of AIV from c. swabs</b>		
Samples tested	62	61
No. Virus Isolated	11	15
<b>Percent Positive</b>	<b>17.7%</b>	<b>24.6%</b>
<b>Detection of AIV after Inoculation in Eggs</b>		
Samples tested	35	34
No. Virus Isolated	7	14
<b>Percent Positive</b>	<b>20.0%</b>	<b>41.2%</b>
Positive in swabs not eggs	1	1
Positive in eggs not swabs	2	7

# Status of Alaskan Samples

USDA AI medium

## Virus recovery in Eggs

Samples Received	525
Samples Tested	170
No. Virus Isolated (HA)	5
<b>Percent Positive</b>	<b>2.9%</b>

## Detection of AIV from c. swabs

Samples tested	78
No. Virus Isolated	2
<b>Percent Positive</b>	<b>2.6%</b>

## Detection of AIV after Inoculation in Eggs

Samples tested	64
No. Virus Isolated	3
<b>Percent Positive</b>	<b>4.7%</b>

Positive in swabs not eggs	2
Positive in eggs not swabs	3

# Concluding Remarks

“The eradication of pathogenic avian influenza viruses seems to be the most effective way to prevent influenza pandemics, although this strategy has not proven successful so far.”

Source: Horimoto and Kawaoka. 2005.

